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G. D. Rosenberg

M. T. Henschen

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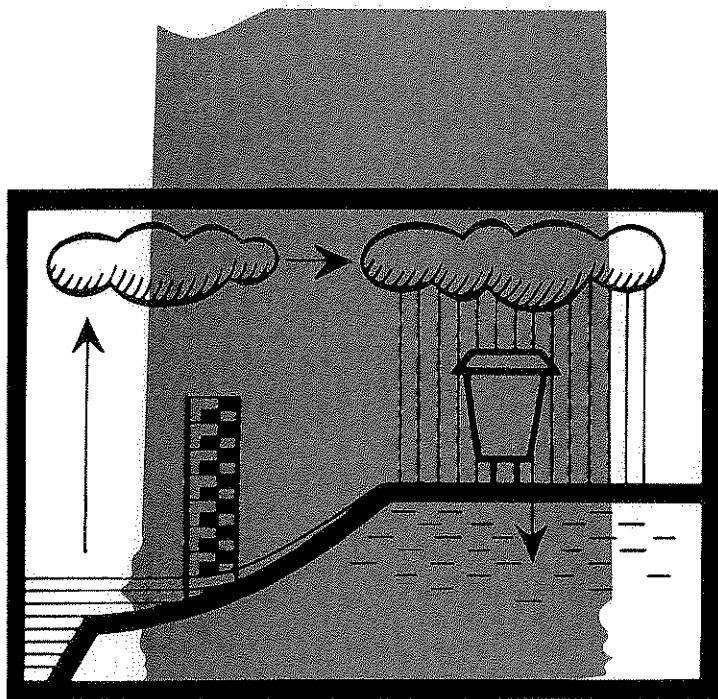
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by

Gary D. Rosenberg
and
Max T. Henschen

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Gary D. Rosenberg and
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Abstract

Exporters of freshwater mussel shells to the Japanese cultured pearl industry report that stains within the inner shell layers are appearing with increasing frequency. Chemical (electron microprobe) analyses of the stains suggest that the stains are due to pollution, and that mussel shells may be useful indicators of water quality.

Although several species of mussels from several Midwestern states (including Indiana, Illinois, Wisconsin, Minnesota, and Iowa) were studied the focus of this report is the Threeridge (Amblema plicata) from the Minnesota--Wisconsin--Iowa area of the Mississippi River between LaCrosse and Prairie du Chien, Wisconsin.

Chemical analyses reveal the presence of aluminum and silicon (clay minerals) in the stains, confirming previous suggestions that mussels are sensitive to turbidity. But the presence of unusual amounts of iron, phosphorus, sulfur, chlorine and perhaps even magnesium, nickel, sodium, and potassium point to the increased deleterious effects that a combination of pollutants can have in a synergistic system. Here, increased turbidity due to increased river traffic or farming combined with metal pollutants results in the metals being concentrated by adsorption to the clay minerals. Grossly unnatural phosphorus and sulfur concentrations in the shell indicate a consequent disturbance of calcium physiology.

Purpose

The purpose of this research was to determine the cause of staining of freshwater mussel shells. In particular, the purpose of this research was to determine whether brown-stained nacre (mother-of-pearl) on the inner surface of freshwater mussel shells is caused by pollution.

The long-term goal of this research is to use mussel shells as bioindicators of different types of pollution, each of which, it is hypothesized, may be recorded in the growth patterns of the shell. The mussel shell is added in growth increments; structurally and perhaps compositionally these increments persist throughout the organism's lifetime as they are not resorbed, and they persist even after death insofar as the shell is not destroyed by weathering. Thus, mussel shells are potentially valuable records of pollution (and other environmental) events even after the organism has died.

It seemed natural to focus on the cause of stained nacre, as the stains appear to be unnatural. At least until recently, pure-white mussel shells were very common in the Midwest and were prized by the Japanese for their cultured pearl industry. Plugs are drilled from the shells, ground into small spheres and inserted into various tissues of marine oysters. The oyster secretes a thin layer of pearl around the "seed" creating a cultured pearl. The "seed" must be white, for any discoloration will show through the transparent outer pearl layers. As the Midwestern United States is underlain by very thick sequences of limestone, midwestern groundwater, lakes, and streams have high concentrations of dissolved calcium. Consequently, midwestern mussel shells long have been known for their thickness and white color.

The potential of molluscan shells as monitors of pollution has not been fully investigated. An enormous amount of research has been devoted to determining the effects of pollution on molluscan soft tissues (Fuller, 1974; Goldberg, 1975), but relatively little work has been done on the relationship between pollution and shell composition.

Yet staining of mussel shell nacre has been known for quite some time. Comments regarding staining of nacre and pearls began to become common in the early 1900's (Wilson and Clark, 1912; Coker, 1915 represent prominent researchers) but reports are known from the 1800's as well (Woodward, 1868).

Thus, even before commencing this research we were faced with several hypotheses for the cause of the staining: (1) staining of mussel shells is not indicative of pollution as stains were present even before pollution of midwestern lakes and streams became severe, (2) pollution of midwestern streams and lakes, due to any number of human activities, became severe enough to affect the molluscan biota (and probably other organisms as well) long before the conservation movement became fashionable in the sixties, (3) stains may be due to different causes, some pollution-related, some not.

We believe that the results of our research support alternatives two and three above. The crux of our argument is that we find several elements in stained mussel shells not commonly found in calcium carbonate skeletons of the mollusca. Moreover, these elements clearly are concentrated at the expense of calcium, so that calcium metabolism is being interfered with adversely.

Materials

Mussels were obtained from several rivers at localities in Indiana, Illinois, Iowa, Wisconsin and Minnesota. However, this report regards only those mussels obtained from 11 localities in Minnesota, Wisconsin and Iowa on the Mississippi River between Prairie du Chien and LaCrosse, Wisconsin. All specimens were obtained live using a crowfoot bar and all reported here were of one commercially important species, the Threeridge or Amblema plicata.

The fact that all specimens of this report were obtained live helps to ensure that the specimens were actually living in the habitats from which they were obtained. Some of the specimens could have been transported downstream by river currents. However, we found no evidence of extensive abrasion of the shells and we submit that use of live specimens at the very least mitigates the problem of transport. Furthermore, use of shells from a one-hundred mile stretch of one river reduces the number of environmental variables that might have complicated matters had we used specimens from widely separated watersheds in different climatic and substrate terrains.

The collecting sites are listed below. The reader will note that two are of particular interest. Locality 20 was just offshore from a metal scrap yard near LaCrosse and Locality 16 was just downstream from a fertilizer plant and sewage treatment plant near Prairie du Chien. Although several mussel species were obtained from each locality, we used only the Threeridge for this report to minimize species-specific effects on shell composition.

- A) Locality 8. Pool 10, Mississippi River mile 642.5. Mid-channel (noncommercial channel), Harper's Slough, approximately 5.5 miles downstream of Lock and Dam 9 and about 3.5 miles south of Harper's Ferry.
- B) Locality 10. Pool 10, Mississippi River mile 636.7, about 300' off the west bank, two miles north of Marquette Island and 1.5 miles south of the mouth of the Yellow River. Approximately one mile above a barge fleeting area.
- C) Locality 11. Pool 10, Mississippi River mile 636.0, about 300' off the west bank, just downstream of a barge fleeting area.

- D) Locality 12. Pool 10, Mississippi River mile 634.7. Beneath Marquette Bridge, in the middle of the main channel.
- E) Locality 13. Pool 10, Mississippi River mile 635.8, east bank, just upstream of Lawler Park, Prairie du Chien. Reportedly, at one time, there was a "metal factory" on the shore at this location.
- F) Locality 14. Pool 10, Mississippi River mile 635.5, east bank, just offshore of Lawler Park, Prairie du Chien.
- G) Locality 15. Pool 10, Mississippi River mile 634.8, west side of east channel, just below lengthy wing dam.
- H) Locality 16. Pool 10, Mississippi River mile 633.5, 300' off the east bank, Pickerel Slough, just upstream of a trailer camp and immediately downstream of sewage and fertilizer plants.
- I) Locality 18. Pool 7, Mississippi River mile 709.0, just north of Dakota, Minnesota, west bank, 200' from shore.
- J) Locality 19. Pools 7/8 (near boundary). Black River at Clinton Street Bridge, LaCrosse, Wisconsin. Black River mile 1.7 = Mississippi River mile 700.5.
- K) Locality 20. Pool 8, Mississippi River mile 698.7. Actually within the Black River at approximately mile 0.5 (i.e. just upstream of Black River and Mississippi River confluence). Adjacent to and downstream from a scrap metal yard.

Methods

After capture, sacrifice, and removal of the soft tissue from the exoskeletons, the shells were prepared for electron microprobe analyses.

Small sections (2 cm in length and width, maximum) were cut using a gemcutter's diamond saw. Sawed sections were generally chosen to include both stained and unstained shell material. The sections were washed and mounted on glass discs with epoxy. For the purposes of this study, unpolished sections were used and the specimens were mounted so that the inner shell surface could be analyzed with the probe. As the microprobe is a surface analyzer, the most recently-deposited stained and unstained nacre were thus analyzed. The specimens were left unpolished to avoid the possibility of contamination before analyses. We recognized that trace and minor elements in skeletal tissues are normally concentrated in amounts sometimes close to the limits of detectability, using energy dispersive microprobe techniques. Thus, we wanted to be certain that all elements recorded during our analyses were actually present in the shell and not introduced during the preparation. This means, however, that the present analyses are strictly semi-quantitative; polished sections are essential to ensure accurate quantitative determinations as topographic variation causes differential absorption of x-rays.

Specimens were analyzed using an ORTEC energy dispersive analyzer (MAC-5 electron microprobe) housed in the Geology Department, IUPUI. Spectra were accumulated for 100 seconds to maximize signal to background ratios. The probe was operated at 10 - 15 kv with a specimen current of .01 μ amps.

Thirty-five specimens were thus analyzed from the habitats previously listed.

Results: Physical Appearance of Stains

Stains are generally brown to yellow or gold in color. They can generally be distinguished from usual color variations of the nacre (which

vary from various shales of purple, peach, rose, etc.) in that they generally have a distinct boundary with unstained shell. Moreover, stains tend to be circular during their early stages of formation and occasionally have small pits in the center with a bit of sediment trapped at the bottom within subsequently-deposited shell. Stains are commonly restricted to the dorsal-most nacre (within the cavity of the umbo), the posterior nacre just dorsal to the pallial line, and (occasionally and less noticeably) ventral to the pallial line. That is, the stains are not randomly smeared across the entire nacre and, when they occur in more than one area do not coalesce unless very severe. The pallial line forms a pronounced boundary between stains when they occur both ventral and dorsal to the line. In a few cases, the stains can be wiped off with the thumb, apparently in the early stages of formation. This, together with the sediments in pits within some stains, clearly suggests that some stains at least are due to very finely entrapped clay material (confirmed with chemical analyses, below). Acetate peels of the shell through stained sections reveal no pronounced disturbance to the structural growth increments unless the stains are severe, in which case a growth break, sometimes filled with sediment can form.

Results: Chemical Analyses

Stoichiometric, pure calcium carbonate contains 40% by weight of calcium. Molluscan shells are commonly stated to be pure calcium carbonate, but they are not (Rosenberg, 1980). They may contain any number of trace and minor elements which vary in concentration along with calcium and the organic matrix. However, few of the trace elements are present in amounts greater than 1%.

In addition to calcium, the stained portions of the mussel shells studied here contain aluminum, silicon, sodium, magnesium, phosphorus, sulfur, chlorine, potassium, iron, and possibly lead, zinc, manganese, mercury, chromium, and silver. Not all stains contain all of these elements but aluminum, silicon, phosphorus, iron, sodium, chlorine, and high concentrations of sulfur are common. (Sulfur is a normal constituent of the organic matrix but, as stated below, concentrations in the stains are unusually high.) Moreover, specimens from Localities 16 and 20 commonly have the highest concentrations of these elements. Recall that Locality 16 is downstream of fertilizer and sewage plants near Prairie du Chien and Locality 20 is near a scrap metal yard at LaCrosse.

Because of our use of unpolished sections, it was not possible to determine percentage concentrations accurately. However, some "order of magnitude" estimates are possible, and some conclusions based strictly on the qualitative determinations are possible:

- A) Stained shells from Localities 16 and 20 commonly have as much as 3% silicon, 6% phosphorus, 10% sulfur, and 2% iron. Calcium is commonly reduced to about 24% in contrast to the normal 40%. These values are grossly unnatural for these key elements.
- B) Calcium is occasionally diminished to a matter of a percent or two in shells from these localities, with simultaneous rises in values for silicon, sulfur, iron, magnesium, chlorine, and phosphorus. The maximum percentages for the latter are uncertain, but the areas of their spectral peaks are commonly many times that for calcium.

- C) Unusual concentrations of the minor elements do occur in specimens from localities other than 16 and 20, but they are not so consistent as in specimens from these two localities. Moreover, they tend to be restricted to the center-most portions (pits) of the stains in localities other than 16 and 20.

Conclusions

We believe that the characteristics of the stains that we have studied suggest that they are aggravated by, if not due solely to, pollution in the Mississippi River system.

The presence of aluminum and silicon in stained portions of the shells clearly confirms the presence of clay minerals observed in some pits in some stains. The source of the clay minerals could be from bottom sediment stirred up by pleasure boats or barges on the Mississippi, or from increasing farming activity along the River, or from effluent from assorted industries and sewage treatment plants.

Stained nacre may well occur even in specimens from unpolluted habitats but (1) the stains are clearly unnatural to the organisms' physiology as they are distributed in the shell with disregard to the usual physiological (allometric) gradients of calcium secretion, (2) the high concentrations of iron in several cases are unlikely due to any but human sources.

It would seem that turbid water with abundant suspended clay minerals by itself can be harmful to mussel physiology, confirming Thiel's (1981) suggestion that the diminishing mussel diversity along the Mississippi River is due to increased siltation following installation of the lock and dam system. We would add the effects of increasing navigation on the River.

But the additional influx of metals (such as iron) combined with the presence of the clays is synergistic as the metals are adsorbed to the clays. The result is a more severely deleterious effect on mussel physiology. We will provide further evidence for this elsewhere.

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